II. REMARKS

Upon entry of the amendment, claims 1, 3-4, and 15-24 will be pending.

Regarding the Amendments

Claim 1 has been amended to replace the phrase "incorporated" with "inserted", *e.g.*, a non-natural amino acid is inserted into a hydrophobic region. In addition, claim 1 has been amended to recite that "the non-natural amino acid causes an increase in the thermal stability of the polypeptide" as compared with a wild-type polypeptide not having the non-natural amino acid. Support can be found at page 8, lines 3- 31 bridging to page 9, lines 1-5; and page 29, lines 20-31 through page 31, including Table 3 in the specification.

Claim 3 has been amended to recite that the non-natural amino acid is "a hydrophobic non-natural amino acid." Support can be found in the specification, especially at page 8, lines 28-31.

Claim 4 has been amended to correct the misspelling of hexafluorovaline.

Claims 15-24 have been added to claim various aspects of the invention. Specifically claim 15 recites that the non-natural amino acid can be a hyper-hydrophobic amino acid. Support for claim 15 can be found at page 7, lines 21-24 in the specification.

Claims 16 and 17 recite that the hydrophobic region of the polypeptide contains at least one leucine, isoleucine, valine, methionine, or phenylalanine. Support can be found at page 11, lines 6-9 in the specification.

Claims 18 and 19 recite the specific insertion sites in a polypeptide for inserting a non-natural amino acid. Support can be found at page 8, line 31, page 9, line 1, and page 11, lines 6-9 in the specification.

Claim 19 recites that the polypeptide contains at least one α-helical structure. Support can be found at page 9, lines 23-31, page 10, line 1-4, and page 11, lines 2-4 in the specification.

Claims 20-23 recite certain features of the hydrophobic region or the polypeptide. Support can be found at page 8, lines 15-24, page 10, lines 29-31, and page 11, lines 1-4 and 25-28 in the specification.

Claim 24 has been added to recite that the polypeptide of the invention can be a leucine zipper polypeptide or a coiled-coil polypeptide. Support for the amendment can be found on page 9, lines 23-31 and throughout the Examples section (e.g., page 28, Example 7) and on page 34 in the Abstract.

These amendments do not add new matter and entry of the amendments is respectfully requested.

Rejection under 35 U.S.C. § 112 second paragraph

The rejection of claims 1-4 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is respectfully traversed.

The Office Action states that claim 3 is unclear with respect to the side chain functionality. To expedite prosecution, Applicant has amended claim 3 to recite the hydrophobic nature of the non-natural amino acid.

The Office Action also states that "it is unclear what constitutes as increased stability" in claim 1. Applicant respectfully points out that the claim has been amended to recite that the increased stability is with respect to a corresponding polypeptide not having a non-natural amino acid inserted. Further, the specification clearly defines the criteria for the improved stability of polypeptides. For example, the specification states that "[i]mproved stability refers to the presence of a higher ratio of folded to unfolded protein relative to that of the wild type protein" and "[i]mproved stability can be determined by examining the amount of folded

protein present under varying conditions of temperature, detergent, and pH." (page 8, lines 5-9; page 29, line 20 bridging to page 31, line 25). Further, increased thermal stability of the polypeptides of the invention is described in the Examples on page 29, line 20 to page 31, line 25.

The Office Action further states that "it is unclear when and how many amino acids are to be substituted for a non-naturally occurring amino acid." Applicant respectfully submits that this issue is moot in light of the amendments made to claim 1, *i.e.,* claim 1 has been amended to recite that at least one non-natural amino acid is inserted into the hydrophobic region of the polypeptide. Further, the Examples of the application show various assays to determine the stability of a polypeptide having insertions of non-natural amino acids, therefore, one of skill in the art can determine whether one or more amino acid residues are required by simple, and not undue, experimentation.

In addition, claim 3 has been amended to be properly dependent on claim 1. Claim 4 has been amended to correct the misspelling of hexafluorovaline.

In view of the amendments made to the claims and the remarks provided above, it is respectfully requested that the rejection of the claims under 35 U.S.C. § 112, second paragraph, be removed.

Rejection under 35 U.S.C. § 112 first paragraph

The rejection of claims 1-4 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was field, had possession of the claimed invention is respectfully traversed.

The Office Action states that the specification does not "sufficiently provide ample definition, such as by structure, formula, or chemical name, of the claimed subject matter sufficient to distinguish it from the desire proteins to other proteins." Applicant respectfully submits that the specification provides ample description of the claimed subject

matter including various characteristics with respect to the structure, formula, and chemical name. For instance, the specification describes various representative leucine zipper and coiled coil polypeptides that can be used as a starting polypeptide to incorporate non-natural amino acids according to the present invention and various representative ways of inserting non-natural amino acids into a desired polypeptide. For example, on page 9 and Examples 4-5, leucine zipper proteins such as GCN4 are exemplified. On page 28, Example 7, a coiled coil protein, A1, is exemplified. On page 26, Example 6, bZip, a leucine zipper and coiled coil protein is exemplified. Support for leucine zipper and coiled coil proteins can be found on page 9, lines 23-31 and throughout the Examples section (e.g., page 28, Example 7) and on page 34 in the Abstract.

According to the specification, the polypeptides targeted for stabilization are those "possessing a hydrophobic core region" and representative examples of these polypeptides include "proteins containing leucine-zipper domains, membrane proteins" and "cytokines such as interleukins, Tumor Necrosis Factor, Granulocyte Colony Stimulating Factor, Erythropoitin, proteases such as Subtilisin, Thermolysin, industrial enzymes such as dehydrogenases, estrases." (page 8, lines 15-18, page 10, lines 29-31 and page 11, lines 1-4).

In addition, the specification states that "[t]he protein site(s) targeted for incorporating non-natural amino acids include region(s) containing hydrophobic amino acids that generally drive protein folding" and representative examples of these hydrophobic amino acids include "leucine, isoleucine, valine, and to a lesser degree methionine and phenylalanine." (page 11, lines 6-9).

According to the specification, "the non-natural amino acid can either replace an existing amino acid in a protein (substitution) or be an addition to the wild type sequence (insertion)" and the incorporation of non-natural amino acids can be accomplished by "known chemical methods including solid-phase peptide synthesis or native chemical ligation, or by biological methods such as, but not limited to, *in vivo* incorporation of the non-natural amino

acid by expression of the cloned gene in a suitable host." (page 8, line 31 and page 9, lines 1-5).

The specification teaches that the incorporation of non-natural amino acids to a hydrophobic region of a polypeptide can increase the stability of the polypeptide and such increased stability of the polypeptide can be determined using various assays described in the specification, *e. g.,* circular dichroism (CD) analysis as described under Example 4 at pages 19 and 20.

The specification also provides various representative examples to illustrate different variance of the claimed polypeptides. Specifically the specification uses two α-helical polypeptides, GCN4-p1 and A1, as representatives of leucine-zipper family and coiled coil proteins. Applicant respectfully points out that the examples of GCN4-p1 and A1 not only provide representative polypeptides but also provides representative examples with respect to the level or amount of incorporating non-natural amino acids in the polypeptides.

In particular, Figure 5 demonstrates various levels of incorporating non-natural amino acids into A1 protein. Specifically the levels of incorporation as shown in Figure 5 include 92%, 76%, 69%, 29%, and 17%. In this experiment, the incorporation is conducted in a random manner with respect to any leucine site, therefore, these specific levels of incorporation should be considered to include various combinations of incorporating non-natural amino acids at the leucine sites, *e.g.,* incorporation should be deemed to have occurred at any one, two, three, four, five, six, or seven of the eight leucine sites in A1 protein (Figure 4A). Example 7 shows the results of stability testing of the three specific levels of non-natural amino acid incorporation, *i.e.,* 92%, 29%, and 17%, and demonstrates that introduction of only a few non-natural amino acids is sufficient to raise the protein folding driving force significantly.

In summary, the specification provides ample description with respect to 1) the polypeptides of different structural characteristics, *e.g.,* leucine-zipper family, membrane proteins, coiled coil proteins, 2) the polypeptides of different functional characteristics, *e.g.,* cytokines, proteases and industrial enzymes, and 3) the polypeptides with various levels of

non-natural amino acid incorporation including incorporation at various hydrophobic amino acid sites. Therefore, the specification provides ample description with respect to various representative subgenuses of the claimed polypeptides and it can be readily appreciated by one skilled in the art that Applicant had possession of the claimed invention at the time the application was filed. In view of the comments above, Applicant respectfully requests withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Rejections under 35 U.S.C. § 102

The rejection of claims 1-4 under 35 U.S.C. § 102(b), as allegedly being anticipated by Rennert et al., Russel et al., Arai et al., and Mendel et al. is respectfully traversed.

The Office Action alleges that claims 1-4 are anticipated by the disclosure of cited prior art. Claim 2 has been cancelled therefore Applicant will address the rejection to pending claims 1, 3 and 4 and new claims 15-23.

Rennert et al., disclose that leucine-requiring mutants of *E. coli* continued to grow if 5',5',5'-trifluoroleucine was added to the medium. Rennert et al. does not specifically teach or disclose a "polypeptide comprising at least one non-natural amino acid inserted into a hydrophobic region of the polypeptide, wherein the non-natural amino acid *causes an increase in the thermal stability* of the polypeptide as compared with a corresponding wild type polypeptide not having the non-natural amino acid" [emphasis added]. In fact, Rennert et al., does not specifically describe any particular protein that incorporates non-natural amino acids, let alone one that specifically exhibits an increase in thermal stability as compared to its wild-type counterpart. Accordingly, since Rennert et al. does not teach each and every element of the pending claims, Applicant respectfully requests that this rejection be withdrawn.

Russell et al., disclose that valine in gramicidin A was chemically replaced by trifluorovaline or hexafluorovaline. Russell et al. does not disclose or describe any proteins that have an increased thermal stability when compared to their wild-type counterpart. Further,

Russell et al., does not teach or describe any proteins, including leucine zipper or coiled-coil proteins, let alone such proteins that have incorporated non-natural amino acids which results in an increase in the thermal stability of the polypeptide as compared with a corresponding wild type polypeptide not having the non-natural amino acid. To Applicants' knowledge, gramicidin A is neither a leucine zipper nor a coiled-coil protein. Therefore, the polypeptides of the present invention are different from the polypeptides disclosed in Russell et al. Accordingly, since Russell et al. does not teach each and every element of the pending claims, Applicant respectfully requests that this rejection be withdrawn.

Arai et al. disclose that two valines were replaced by L-hexafluorovaline (Hfv) residues via solid-phase-synthesis and cyclization-cleavage method in gramicidin S. Thus, Russell et al. does not disclose or describe any proteins, including leucine zipper or coiled coil proteins, that have incorporated non-natural amino acids which results in an increase in the thermal stability of the polypeptide as compared with a corresponding wild type polypeptide not having the non-natural amino acid. Therefore, the polypeptides of the present invention are different from the polypeptides disclosed in Arai et al. Accordingly, since Arai et al. does not teach each and every element of the pending claims, Applicant respectfully requests that this rejection be withdrawn.

Mendal et al. disclose the replacement of a leucine with S,S-2-amino-4-methylhexanoic acid at position 133 in T4 lysozyme. Mendal et al. does not disclose or describe any proteins, including leucine zipper or coiled-coil proteins, let alone such proteins that have incorporated non-natural amino acids which results in an increase in the thermal stability of the polypeptide as compared with a corresponding wild type polypeptide not having the non-natural amino acid. Therefore, the polypeptides of the present invention are different from the polypeptides disclosed in Mendal et al. Accordingly, since Mendal et al. does not teach each and every

element of the pending claims, Applicant respectfully requests that this rejection be withdrawn.

In view of the comments above, Applicant respectfully requests withdrawal of the rejections under 35 U.S.C. 102(b).

Applicants submit that claims 1, 3, 4 and 15-24 are in condition for allowance. In view of the foregoing amendment and remarks, it is believed that the Examiner should withdraw the rejections of the pending claims. The Examiner is invited to call Applicants' representative at the number below to expedite allowance of the pending claims if there are any questions.

The Commissioner is hereby authorized to charge any other fees that may be associated with this communication, or credit any overpayment to Deposit Account No. 50-1355.

Respectfully submitted,

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